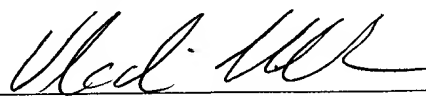


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Attorney Docket No: GALO-007/01US

PATENT

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 on June 18, 2001.

By: 
Vladimir Skliba

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of Robert O. Messing, et al.

Serial No.: 09/340,283

Examiner: Ram Shukla

Filed: June 25, 1999

Art Unit: 1632

For: PROTEIN KINASE C EPSILON AS MODULATOR OF ANXIETY,
ALCOHOL CONSUMPTION AND SELF ADMINISTRATION OF DRUGS OF
ABUSE

Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION UNDER 37 CFR 1.132

The undersigned, Robert O. Messing, M.D., does hereby declare and state that:

1. I am an inventor of the above-referenced application U.S.S.N. 09/340,283, filed June 25, 1999, based on U.S. provisional applications U.S.S.N. 60/091,755, filed July 6, 1998, and U.S.S.N. 60/125,995, filed March 24, 1999. Along with my co-inventor Clyde O. Hodge, Ph.D., I performed or directed all of the experiments described and claimed in these applications.

Consented Don 9/5/01

2. PKC ϵ null mice appeared less anxious than wild type mice since they spent significantly more time in the center of the open field than did wild type mice in locomotor chambers. (See Fig. 7 in specification of above-referenced application.) In preliminary work, reduced anxiety was also apparent on the elevated plus maze, where male PKC ϵ null mice spent more time ambulating and resting, and traveled greater distances on the open arms of the maze. (See Fig. 9 in application.) In contrast, no differences were observed for female mice. The elevated plus maze is a well-validated model for testing anxiety in male rodents. [S. Hogg, *Pharmacol. Biochem. Behav.* (1966) 54:21-30; copy attached] It has been used in female rodents, but there is uncertainty about its validity in females [G. G. Nomikos and C. Spyraiki, *Neuropharmacology*, (1988) 27:691-696; copy attached]. Therefore we restricted all subsequent studies to male mice.

3. On further testing, we once again found evidence for reduced anxiety in male PKC ϵ null mice (see Fig. 1 below). This behavior was apparent for C57Bl/6 x 129SvJae hybrid mice and inbred 129SvJae mice, despite striking differences due to genetic background. These findings indicate that loss of PKC ϵ is associated with reduced anxiety and can be observed despite differences in genetic background in different mouse strains.

We did this experiment because all prior data was obtained in hybrid mice. We wanted to confirm that the phenotype was not due to segregation of other 129SvJae and C57 genes in wild-type versus knock-out mice. In the 129SvJae inbreds, the *only* difference between wild-types and knock-outs is at the PKC ϵ gene locus, so the difference in behavior seen is due to the PKC ϵ null mutation, not to some spurious segregation of alleles of other genes.

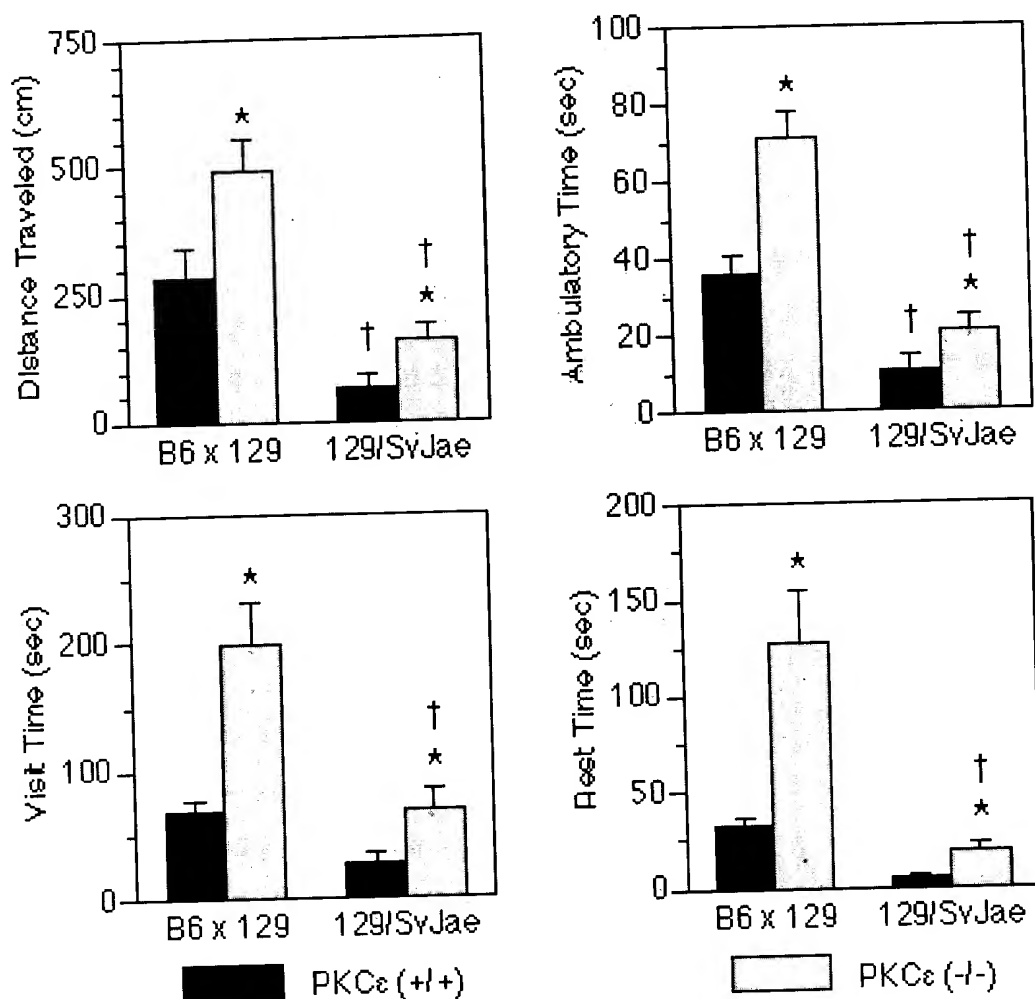


Fig. 1. Behavior of wild type (black bars, n=8) and PKCε null (gray bars, n=8) mice after placement in a novel plus maze for 5 min. PKCε null mice traveled and rested more in the opens arms of the maze regardless of mouse strain [$F_{1,27}$ (genotype)=13.9, $p=0.001$; ($F_{1,27}$ (strain)=34.6, $p<0.001$; $F_{1,27}$ (genotype x strain), NS]. * $p < 0.05$ compared with wild type, † < 0.05 compared with B6x129 hybrid mice of same PKCε genotype (Tukey post hoc test). No differences were observed for these parameters in the closed arms (not shown).

DECLARATION

I declare that all statements made here of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date:

6/18/01

By:

Robert O. Messing

Robert O. Messing, M.D.